In the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Upon entry of the present amendment, the claims will stand as follows:

Please cancel claim 2 without prejudice.

Please amend claims 1, 3, 9, 10, 14-16, 24, 25, 43, 46, 47, and 55 as follows:

1. (Currently Amended) A method for identifying an essential chromosomal gene in a haploid test organism, said method comprising:

constructing a bacterial artificial chromosome (BAC)-carrying merodiploid test cell by transforming a wild-type haploid host cell whose genomic sequence is known, which is naturally capable of being transformed ~~by artificial means~~ and ~~is capable of~~ undergoing DNA recombination, with a ~~bacterial artificial chromosome (~~BAC[[)]] carrying a known segment of DNA of the haploid test organism with about 80% to 100% sequence identity to a known segment of chromosomal DNA in the host cell, and wherein replication of the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the host cell;

inserting randomly a bacterial transposon into the merodiploid test cell so as to disrupt function of a gene therein;

culturing one or more of the BAC-carrying merodiploid test cells in a suitable culture medium while introducing the environmental condition so as to transform the merodiploid test cells into haploid test cells; ~~and~~

identifying one or more of the haploid test cells that contain transposon-mutagenized DNA in an essential chromosomal gene therein, and

obtaining the identity of the essential chromosomal gene in the test organism by locating a gene in the known segment of DNA of the haploid test organism that contains the transposon-mutagenized DNA.

Claim 2. (Cancelled)

3. (Currently Amended) The method of claim 1 wherein ~~the identifying~~ obtaining the identity of the essential chromosomal gene involves selection of test cells that do not survive subjection to the environmental condition as having the transposon in an essential chromosomal gene therein.

4. (Original) The method of claim 1, wherein the transposon is Tn5 or Tn10.

5. (Original) The method of claim 4, wherein the transposon is operatively linked to a first antibiotic resistance gene.

6. (Original) The method of claim 5, wherein the BAC comprises a second antibiotic resistance gene, wherein the first and second antibiotic resistance genes convey resistance to two different antibiotic compounds.

7. (Previously Presented) The method of claim 6, wherein the first and second antibiotic resistance genes are selected to provide resistance to a pair of antibiotics selected from the group consisting of ampicillin, tetracycline, kanamycin, and chloramphenicol.

8. (Previously Presented) The method of claim 7, wherein the first and second antibiotic resistance genes provide resistance, respectively, to kanamycin and chloramphenicol.

9. (Currently Amended) The method of claim 6, wherein ~~the~~ identifying one or more of the haploid test cells that contain transposon-mutagenized DNA in an essential chromosomal gene therein includes subjecting the test cells to both of the antibiotics to which the first and second antibiotic resistance genes provide resistance.

10. (Currently Amended) The method of claim 1, wherein the BAC is temperature sensitive for replication and the environmental condition is a temperature that is selectively non-permissive for replication of the BAC in the test cell.

11. (Original) The method of claim 1, wherein the BAC is suppressor sensitive for replication and the environmental condition is a suppressor that selectively suppresses replication of the BAC in the test cell.

12. (Original) The method of claim 1, wherein the host cell is selected from the group consisting of *E. coli, Salmonellae,* and *B. subtilis.*

13. (Original) The method of claim 12, wherein the host cell is *E. coli.*

14. (Currently Amended) The method of claim 1, wherein the identified essential chromosomal gene has 100% sequence identity with a gene in the known segment of DNA [[in]] from the [[host cell]] haploid test organism.

15. (Currently Amended) The method of claim 1, wherein the identified essential chromosomal gene has at least 90% sequence identity with a gene in the known segment of DNA [[in]] from the [[host cell]] haploid test organism.

16. (Currently Amended) The method of claim 1, wherein the identified essential chromosomal gene has at least 80% sequence identity with a gene in the known segment of DNA [[in]] from the [[host cell]] haploid test organism.

17. (Original) The method of claim 1, wherein the BAC contains up to 100 genes of the haploid test organism.

18. (Original) The method of claim 1, wherein the haploid test organism and the host cell are the same species of prokaryote.

19. (Original) The method of claim 1, wherein a library of the BAC-carrying merodiploid test cells is constructed such that the BACs in the library collectively contain the entire genome of the haploid test organism.

20. (Original) The method of claim 19, wherein the entire genome of the haploid organism is contained in about 50 to 100 merodiploid test cells that each contain a unique segment of the genome of the haploid test organism.

21. (Original) The method of claim 19, wherein the test cells in the library are simultaneously subjected to the environmental condition.

22. (Original) The method of claim 1, wherein sufficient of the merodiploid test cells are constructed to provide four-fold coverage of the entire genome of the haploid organism.

23. (Original) The method of claim 1, wherein the BAC in the merodiploid test cell is contained within a fosmid/cosmid.

24. (Currently Amended) The method of claim 23, wherein the fosmid/cosmid is packaged in lambda phage prior to ~~insertion into the merodiploid test cell~~ transformation of the haploid host cell.

25. (Currently Amended) A method of screening compounds for putative antibiotic activity against a pathogenic bacterium whose genome is known, said method comprising:

constructing a BAC-carrying merodiploid test cell by transforming a wild-type haploid host cell whose genomic sequence is known, which is naturally capable of being transformed ~~by artificial means~~ and undergoing DNA recombination with a BAC that carries a known segment of DNA of a haploid pathogenic bacterium, and wherein the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the test cell;

inserting randomly a transposon into the merodiploid test cell so as to disrupt function of a gene therein;

culturing one or more of the merodiploid test cells in a suitable culture medium while introducing the environmental condition;

identifying one or more test cells that do not survive subjection to the environmental condition as containing the transposon in an essential chromosomal gene therein;

locating the essential gene in the ~~known segment of DNA of the~~ pathogenic bacterium by identifying a gene in the known segment of DNA of the pathogenic bacterium inserted into the [[haploid]] test cell by the BAC that has been disrupted by the transposon; and

screening the essential gene from the pathogenic bacterium or a bacterial protein encoded by the essential gene against putative antibiotic compounds to determine those compounds that bind to or interrupt function of the essential gene or the bacterial protein, wherein such a compound is a candidate antibiotic against the pathogenic bacterium.

26. (Original) The method of claim 25, wherein the transposon is Tn5 or Tn10.

27. (Original) The method of claim 25, wherein the transposon is operatively linked to a first antibiotic resistance gene.

28. (Original) The method of claim 27, wherein the BAC comprises a second antibiotic resistance gene.

29. (Previously Presented) The method of claim 28, wherein the first and second antibiotic resistance genes are selected to provide resistance to a pair of antibiotics selected from the group consisting of ampicillin, tetracycline, kanamycin, and chloramphenicol.

30. (Previously Presented) The method of claim 29, wherein the first and second antibiotic resistance genes provide resistance, respectively, to kanamycin and chloramphenicol.

31. (Previously Presented) The method of claim 25, wherein the locating includes subjecting the test cells to the antibiotics to which the first and second antibiotic resistance genes provide resistance.

32. (Original) The method of claim 25, wherein the BAC is temperature sensitive and the environmental condition is a non-permissive temperature for replication of the BAC in the test cells.

33. (Original) The method of claim 25, wherein the BAC is suppressor sensitive and the environmental condition is a suppressor that selectively prevents replication of the BAC in the test cells.

34. (Original) The method of claim 25, wherein the host cell is selected from the group consisting of *E. coli, Salmonellae, and B. subtilis.*

35. (Original) The method of claim 34, wherein the host cell is *E. coli.*

36. (Original) The method of claim 25, wherein the BAC contains up to 100 genes of the pathogenic bacterium.

37. (Original) The method of claim 25, wherein a library of the BAC-carrying merodiploid test cells is prepared such that the BACs in the library collectively contain the entire genome of the pathogenic bacterium.

38. (Original) The method of claim 37, wherein the entire genome is contained in about 50 to 100 merodiploid test cells that each contain a unique segment of the genome of the pathogenic bacterium.

39. (Original) The method of claim 38, wherein the test cells in the library are simultaneously subjected to the environmental condition.

40. (Original) The method of claim 25, wherein the candidate antibiotic is bactericidal.

41. (Original) The method of claim 25, wherein the bacterium is pathogenic in at least one mammalian species.

42. (Original) The method of claim 25, wherein the bacterium is pathogenic in at least one plant species.

43. (Currently Amended) A method for identifying an essential chromosomal gene in a haploid test organism, said method comprising:

constructing a BAC carrying a known segment of DNA of the haploid test organism with about 80% to 100% sequence identity to a known segment of chromosomal DNA in a haploid host cell having a known genomic sequence, which is naturally capable of being transformed ~~by artificial means~~ and undergoing DNA recombination with the BAC;

inserting randomly a bacterial transposon into the BAC so as to disrupt function of a gene in the segment of chromosomal DNA;

introducing the BAC into the haploid host cell to create a merodiploid test cell;

culturing the merodiploid test cell in a suitable culture medium ~~such that~~ while introducing an environmental condition ~~to which the BAC in the test cell is sensitive to an environmental condition~~ that selectively prevents replication of the BAC in the test cell;

identifying one or more BAC-carrying merodiploid test cells that do not survive in culture as containing the transposon in an essential chromosomal gene therein; and

obtaining the identity of the essential chromosomal gene by determining which gene in the known segment of DNA of the haploid test ~~cell~~ organism inserted into the BAC was disrupted by the transposon.

44. (Original) The method of claim 43, wherein the transposon is Tn5 or Tn10.

45. (Original) The method of claim 43, wherein the transposon is operatively linked to a first antibiotic resistance gene.

46. (Currently Amended) The method of claim 43, wherein the transposon is inserted randomly into the BAC *in vitro* prior to introduction of the BAC into the ~~test~~ haploid host cell.

47. (Currently Amended) The method of claim 46, wherein ~~the known segment of DNA~~ the BAC is linearized prior to introduction into the host cell.

48. (Original) The method of claim 43, wherein the host cell is an *E. coli* RecBC-SbcBC quadruple mutant.

49. (Original) The method of claim 43, wherein the identifying includes subjecting the test cells to the antibiotic to which the antibiotic resistance gene provides resistance.

50. (Original) The method of claim 43, wherein the host cell is selected from the group consisting of *E. coli, Salmonellae,* and *B. subtilis.*

51. (Original) The method of claim 43, wherein the BAC contains up to 100 genes of the test organism.

52. (Original) The method of claim 43, wherein a library of the BAC-carrying merodiploid test cells is prepared such that the BACs in the library collectively contain the entire genome of the test organism.

53. (Original) The method of claim 52, wherein the entire genome is contained in about 50 to 100 merodiploid test cells that each contain a unique segment of the genome of the test organism.

54. (Original) The method of claim 43, wherein the test organism is a pathogenic bacterium.

55. (Currently Amended) The method of claim 54, wherein the method further comprises screening [[an]] the essential gene ~~obtained~~ from the pathogenic bacterium or a bacterial protein encoded thereby against putative antibiotic compounds to determine those compounds that bind to or interrupt function of the ~~corresponding~~ essential gene or the bacterial protein, wherein such a compound is a candidate antibiotic against the pathogenic bacterium.